UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/580,024	09/05/2006	Alexander Ludemann	028622-0153	7531

22428 7590 10/27/2009

FOLEY AND LARDNER LLP
SUITE 500
3000 K STREET NW
WASHINGTON, DC 20007

EXAMINER	
KINGAN, TIMOTHY G	

ART UNIT	PAPER NUMBER
1797	

MAIL DATE	DELIVERY MODE
10/27/2009	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/580,024	LUDEMANN ET AL.	
	Examiner	Art Unit	
	TIMOTHY G. KINGAN	1797	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *22 June 2009*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-23,25 and 29* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-23,25 and 29* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *03/16/2009 and 08/25/2009*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Response to Arguments

1. Applicant's arguments filed 06/22/2009 have been fully considered but they are not persuasive.

In evaluating Lee and its relevance to the instant claims, all of the teachings of the reference are determined and evaluated in a manner to include what they would suggest to one of ordinary skill in the art. Individual teachings of the reference, or even those emphasized, are not taken in isolation or evaluated in piecemeal fashion.

Applicant's method of claim 1 requires only that cells take up a labeled compound such that its metabolites are saturated with the isotope of the compound. Since ^{12}C is an isotope of carbon, the claim as written may require revision to clarify applicant's intent.

Nevertheless, conventional usage of the term "saturated" indicates that all atoms of an individual element in a compound are present as an isotope different from the most abundant form found in nature, e.g., the substitution of ^{13}C for ^{12}C in a metabolic precursor. In [0073], Lee teaches "The precursor molecule can be any molecule which normally contains a ^{12}C. Further, 1, 2, 3, 4, 5, 6 or any number of ^{13}C labels can be included within the precursor molecule. An example of a precursor molecule typically utilized in connection with the invention is a glucose molecule." Since glucose has 6 carbons atoms, such teaching suggests, and it would be obvious to one of ordinary skill in the art of metabolic studies, the step of using uniformly labeled glucose in the method of labeling metabolites. From there, one of ordinary skill in the art would recognize that,

since cellular compounds turn over metabolically, and with different rates, that it would be possible, in labeling for a sufficient period of time, to saturate all metabolites with the label: glucose, for instance, is, eventually, completely metabolized to CO_2, but a number of sugars, amino acids, proteins, etc. are labeled along the way. Therefore, applicant is incorrect in asserting that the teachings of Lee do not suggest saturation labeling. The collection of metabolites is known as the "metabolome" (see [0028] in Lee). Together with the teaching of Lee comprising liquid or gas chromatography interfaced with mass spectrometry, the resolution of which may be 1 Dalton or less, the reference teaches or suggests the elements of applicant's method.

In further response, examiner notes that nothing in Lee's method requires the period of time in metabolizable compound be confined to use of ^{13}C-labeled material. The teaching of Lee on mass isotopomers, together with the teaching of uniformly-labeled precursor (above; and see rejection statements), would suggest to one of ordinary skill in the art the desirability of labeling to saturation followed by removal of ^{13}C-label and addition of ^{12}C-labeled compound in order to determine metabolic alterations in specific pathways, for instance to serve studies of disease states or the actions of pharmaceuticals. Applicant is incorrect in asserting that saturation labeling "would make no sense in the method of Lee" (p. 9); Lee teaches methods of studying metabolism and that precursors may be partially or uniformly labeled. Precursors are metabolized, whether they are partially or uniformly labeled, and metabolites can be detected and quantified in analysis of isotopomers, which is also taught by Lee.

With regard to applicant's arguments that the secondary references do not remedy the deficiencies of Lee, examiner notes that applicant suggests different reasons for combining than those used in the office action of 12/23/2008. The bases for the citations are found in the office action and are relevant to secondary elements; they were not used for documentation of saturation labeling.

Claim Rejections - 35 USC § 103

1. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

2. **Claims 1, 5, 6, 7, 8, 10, 11, 12, 13, 15 and 20-22** are rejected under 35 U.S.C. 103(a) as being unpatentable over W-N.P. Lee and L.G. Boros, U.S. Patent Application Publication 2003/0180710 (herein after Lee).

For Claims 1, 6-8, 10-13 and 15, Lee teaches methods for determining metabolic processes associated with glucose metabolites (abstract) in which precursor ^{13}C labeled glucose, labeled at specific positions (abstract) or containing any number of ^{13}C labels, up to labels at all carbons (6 atoms of ^{13}C; U-^{13}C-glucose) [0073], is provided to cells in culture (allowing uptake of stable isotope) for the purpose of creating an information profile of metabolism including metabolic pathway flow, specific metabolite synthesis patterns, rate of metabolite synthesis, contribution of individual synthetic reactions [0017] and analyzing the stable isotope labeled metabolome [0028] by producing intermediary metabolites from labeled precursor, including sugar phosphates, metabolites of the pentose cycle, glycogen, TCA cycle intermediates, glycolytic

intermediates and fatty acids [0089]. Lee also teaches mass isotopomers of these metabolites which can be separated (fractionated or purified) by liquid and gas chromatography and then measured and quantified by mass spectrometry [0032] (metabolites chromatographically separated to create a subset of metabolites prior to quantitative determination; fully capable of identification of metabolite for quantitation based on chromatographic behavior and pairing of m/z peaks in MS), including MALDI-TOF [0036]. Lee is silent on culture conditions leading to saturation of metabolites with isotope. Lee does teach samples are taken from labeled cells at multiple times [0077]. It would have been obvious to one of ordinary skill in the art from such teaching of Lee to use a sampling of labeled cells after a time consistent with saturation of metabolites with labeled isotope in order to provide for a consistent and maximum possible resolution in MS of such isotopically labeled metabolites from unlabeled metabolites from control cells.

For Claim 5, Lee does not teach the number of metabolites which are quantitatively determined. It would have been obvious to one of ordinary skill in the art, from the teaching of Lee on the number and complexity of metabolic pathways accessed by labeling with ^{13}C-glucose, to monitor and quantify at least 50 metabolites in order to provide a method capable of revealing defects in the maximum number of enzymatic pathways.

For Claim 9, Lee teaches the system under study may comprise plant or animal cells [0079].

For Claim 20, Lee teaches use of ^{13}C-labeled precursor, in labeling cells in culture according to claim 1, for measuring metabolic enzyme levels as biomarkers for disease processes ([0017, Fig. 2) or DNA or RNA [0053] (transcripts).

For Claim 21, Lee does not teach use of the same biological sample for metabolites and protein and/or transcript determinations. Lee does teach use of labeled and treated cultures for metabolome studies (determination of metabolites) followed by proteomic or genetic studies ([0052, Fig. 2) (determination of proteins or transcripts). It would have been obvious to one of ordinary skill in the art to use the same cultures for metabolite and protein or nucleic acid studies in order to make most efficient use of cultured material as well as to provide an optimal basis for comparing and correlating results from metabolite studies with those from protein or nucleic acid studies.

For Claim 22, Lee does not teach statistical evaluation of data. Lee does teach the relevance to and applications in metabolite monitoring for Phase I, II and III trials in getting approval from the FDA for treatments in new drug applications [0085]. It would have been obvious to one of ordinary skill in the art, from such suggestion of Lee, to use appropriate and industry- and government agency-recognized statistical analysis of variance of data in order to provide for assigning levels of confidence to any conclusions based on the data, the results of such analysis potentially being critical in conclusions of efficacy.

3. **Claims 2-4** are rejected under 35 U.S.C. 103(a) as being unpatentable over Lee in view of F.P. Abramson and P. Lecchi, U.S. Patent Application Publication 2003/0077572 (herein after Abramson).

For Claim 2, Lee does not teach combining biological samples with the first sample being either unlabeled or labeled differently from the second sample. Abramson teaches methods for quantitatively monitoring components (metabolites) of a cell population, in which growing cells may be provided with ^{13}C-glucose as sole carbon source, such monitoring being done by determination of isotope ratio in a mass spectrometer ([0016]; abstract). Further, Abramson teaches combining control cells (unlabeled) with cells labeled with stable isotopes followed by detecting quantitative isotopic enrichment by ratio-monitoring [0024]. It would have been obvious to one of ordinary skill in the art from such teachings to use the well known approach of combining, prior to analysis, separate labeled and unlabeled extracts, or cells prepared in the presence or absence of labeled metabolic precursor, in order to attain the advantages of internal standards in which control and test materials are treated identically during analysis, thereby providing straight-forward comparisons including corrections for recovery.

For Claims 3 and 4, Lee does not teach first and second samples corresponding to different phenotypes or genotypes. Abramson teaches use of a genetically modified variant of the original cell for labeling and combination with control cells (first and second samples are different genotype, i.e., a transgenic by virtue of the genetic modification in the variant). It would have been obvious to one of ordinary skill in the art

to use genetic variants in comparisons of metabolites, according to the teaching of Abramson and in the method of Lee, in order to provide an analysis, different from that of gene expression studies, that could provide a more comprehensive view of metabolic consequences of genetic lesions beyond that mediated by a single enzyme to possible secondary pathways, according to the teaching of Lee [0004].

4. **Claim 14** is rejected under 35 U.S.C. 103(a) as being unpatentable over Lee in view of S. Kasper, U.S. Patent Application Publication 2005/0112706 (herein after Kasper).

For Claim 14, Lee does not teach introducing external standards for one or more metabolites. Examiner notes that once a standard is "introduced" into a sample prior to analysis, it is commonly known as an "internal" standard (standard and sample metabolite intimately mixed), while an external standard is analyzed separately from metabolite in sample. The use of such standards is known in the art. Kasper teaches analysis of biologically relevant metabolites by MALDI-TOF-MS in which internal standards (external standard added to sample) is employed, the preferred such standard being a stable isotope labeled internal standard (isotopomers) [0055]. It would have been obvious to one of ordinary skill in the art to use the standards of Kasper in the method of Lee in order to provide for correction of recovery in quantitative studies as well as to provide for isotopomers of metabolites that facilitate identification of sample metabolites based on detection of mass pairs.

5. **Claims 16 and 17** are rejected under 35 U.S.C. 103(a) as being unpatentable over Lee in view of C. Birkemeyer et al., J. Chromatography A 993:89, 2003 (herein after Birkemeyer).

For Claims 16 and 17, Lee does not teach identification of metabolites by secondary fragmentation. Such method of identification, made possible by predictable patterns of fragmentation in MS, is known in the art. Birkemeyer, teaches derivatization of phytochromes, including acidic compounds, by silylation (p. 90, ¶ 4), fragmentation of such derivatives in MS and identification by comparison of measured m/z values with data found in the literature as well as in a commercial mass spectral library (p. 92, ¶ 9). Further, Birkemeyer teaches detection of fragments by GC-MS with electron impact ionization and monitoring of total or selected fragment ions (p. 93, ¶ 2). It would have been obvious to one of ordinary skill in the art to use derivatization and identification of selected derivatives by secondary fragmentation in MS, according to Birkemeyer and in the method of Lee, in order to make use of established methods for preparation and analysis of metabolites that provide for increased sensitivity associated in part with increased volatility and, therefore, recovery of analyte in detection.

6. **Claims 18-19** are rejected under 35 U.S.C. 103(a) as being unpatentable over Lee in view of M.K. Hellerstein and R.A. Neese, Am. J. Physiol. Endocrinol. Metab. 276:1146-1170, 1999 (herein after Hellerstein-APEM).

For Claims 18-19, Lee does not teach uptake of unlabeled compounds in cells cultured before or after in isotope labeled compound. Lee does teach that ^{13}C labeled

compounds such as glucose may substitute for compounds which normally contain ^{12}C isotope, in functioning as precursors added to a changing test system, such as cells in culture [0083]-[0084]. The sequential exposure of cells to labeled and unlabeled compounds, in vivo or in vitro, with time-dependent measurement of isotope in metabolites comprises a "pulse-chase" experiment and is known in the art. Hellerstein-APEM teaches such experiments with stable isotope for quantifying by mass spectrometry relative abundances of molecular species of polymeric isotopomers (abstract, Table 1) (metabolites). It would have been obvious to one of ordinary skill in the art, from such teachings of Hellerstein-APEM to use such sequential culture in the method of Lee in order to provide the opportunity to obtain time-dependent estimates (kinetic) of turnover of metabolites. Further, the comparison of metabolite distribution following a "chase" period with the distribution before the chase (without uptake of unlabeled compound) comprises an inclusion of a data point at time 0 and would have been obvious to one of ordinary skill in the art as essential for meaningful and comprehensive kinetic determinations of isotope distribution.

7. **Claim 25** is rejected under 35 U.S.C. 103(a) as being unpatentable over Lee in view of M.K. Hellerstein, U.S. Patent Application Publication 2004/0081994 (herein after Hellerstein-'994).

For Claim 25, Lee does not teach a kit comprising an isotopically labeled compound and a manual. Such kits are known in the art. Hellerstein-'994 teaches a kit comprising isotopically labeled precursor molecules and instructions (a manual) [0016]

for use in biochemical methods for measuring synthesis and turnover of molecular components (abstract). It would have been obvious to one of ordinary skill in the art to prepare a kit comprising one or more labeled metabolites, according to the teaching of Hellerstein-'994 and in the method of Lee, in order to provide the convenience of premeasured and quantified reagents for use as internal standards as well as to provide established and tested protocols for use of such reagents.

8. **Claim 23** is rejected under 35 U.S.C. 103(a) as being unpatentable over Lee in view of L.T. Evans et al., U.S. Patent 5,532,206 (herein after Evans).

For Claim 23, Lee teaches methods for determining metabolic processes associated with quantification of glucose metabolites in cells labeled with precursor ^{13}C labeled glucose (abstract); Lee does not teach a set of isotopically labeled metabolites obtainable from such labeled cells. Such compounds labeled with stable isotopes (metabolites) are known in the art for use as internal standards for identification. Evans teaches stable isotope-labeled gibberellin GA1 (metabolite) and its use as an internal standard in quantifying GA1 in mass spectrometry, a metabolite of applied GA20 (col 20, lines 65-67). It would have been obvious to one of ordinary skill in the art to prepare a set of labeled compounds, according to the teaching of Evans and representative of one or more (a set) of metabolites of an applied precursor in order to facilitate the process of identification of said metabolites from cell extracts by mass pairing in the process of detection and their quantification by use of known amounts of such standards in mixing with cell extracts.

9. **Claim 29** is rejected under 35 U.S.C. 103(a) as being unpatentable over Lee in view of Evans as applied to claim 23 above, and further in view of Hellerstein-'994.

For Claim 29, Lee, Evans and Hellerstein-'994 do not teach a kit of isotopically labeled metabolites. Hellerstein-'994 teaches a kit comprising isotopically labeled precursor molecules [0016]. It would have been obvious to one of ordinary skill in the art to include, in such kit, isotopically labeled metabolites representative of metabolic products of the precursor of Hellerstein-'994, in order to facilitate the process of identification of precursor metabolites from cell extracts by mass pairing in the process of detection and their quantification by use of known amounts of such standards in mixing with cell extracts.

Conclusion

1. **THIS ACTION IS MADE FINAL.** Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of

the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to TIMOTHY G. KINGAN whose telephone number is (571)270-3720. The examiner can normally be reached on Monday-Friday, 8:30 A.M. to 5:00 P.M., E.S.T..

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jill Warden can be reached on 571 272-1267. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

TGK

/Jill Warden/
Supervisory Patent Examiner, Art Unit 1797